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2.	Patent application number *(The Patent Office will fill in this part)*	29 MAR 2003 0307366.5
3.	Full name, address and postcode of the or of each applicant *(underline all surnames)*	MITSUBISHI PHARMA CORPORATION 2-2-6, Nihonbashi-Honcho Chuo-ku Tokyo 103-8405 JAPAN
	Patents ADP number *(if you know it)*	08438616002
	If the applicant is a corporate body, give the country/state of its incorporation	
4.	Title of the invention	Compounds
5.	Name of your agent *(if you have one)*	CRUIKSHANK & FAIRWEATHER
	"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*	19 Royal Exchange Square Glasgow G1 3AE UNITED KINGDOM
	Patents ADP number *(if you know it)*	547002

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Signature Cruikshank & Fairweather Date 28/3/03

CRUIKSHANK & FAIRWEATHER 28TH MARCH 2003

12. Name and daytime telephone number of person to contact in the United Kingdom DR. P. CHAPMAN - 0141-221-5767

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DUPLICATE

COMPOUNDS

Technical Field

The present invention relates to novel treatments for schizophrenia, based on the concept of identifying agents capable of selectively binding to the serotonin 5-HT7 and muscarinic m4 receptors and the use of such compounds in treating schizophrenia.

Background Art

The antipsychotic drugs (APDs) currently used in the treatment of schizophrenia are less than optimal in many respects, showing a lack of efficacy against some of the symptoms of schizophrenia and a significant tendency to produce unpleasant side-effects. While all APDs are effective against the positive symptoms of schizophrenia in the majority of patients, they are all less than completely effective against the negative symptoms and cognitive deficits of the disease, with many APDs showing virtually no efficacy against these symptoms. Negative symptoms include loss of emotional responsiveness, lack of motivation and social withdrawal. Cognitive deficits include deficits in working memory, attention and executive function. In addition, in a significant proportion of patients, the positive symptoms which include hallucinations and delusions do not respond to conventional antipsychotic drugs. All current APDs share the common property of affinity and antagonist action at D2 dopamine receptors (Seeman, 2001). This is thought to

underly their activity against the positive symptoms, but unfortunately is responsible also for unpleasant side-effects such as parkinsonian motor deficits and hyperprolactinaemia.

Hence there is an urgent need for effective APDs which are able to ameliorate both positive and negative symptoms and the cognitive deficits of schizophrenia without significant D2 affinity.

Disclosure of Invention

It is an object of the present invention to provide a novel pharmaceutical agent which combines serotonin 5-HT7 receptor antagonist activity and muscarinic m4 receptor agonist activity for use in the treatment of schizophrenia and/or bipolar disorder.

It is a second object of the present invention to provide the abovementioned pharmaceutical agents which additionally possess relatively low or negligible dopaminergic D2 affinity which are useful as antipsychotic agents useful for the treatment of schizophrenia and/or bipolar disorder.

It is a third object of the present invention to provide an agent which represents a novel class of antipsychotic drug, useful for the treatment of schizophrenia and/or bipolar disorder.

It is yet a further object of the present invention to provide an agent according to the third object which additionally possess relatively low or negligible dopaminergic D2 affinity which represents a novel class

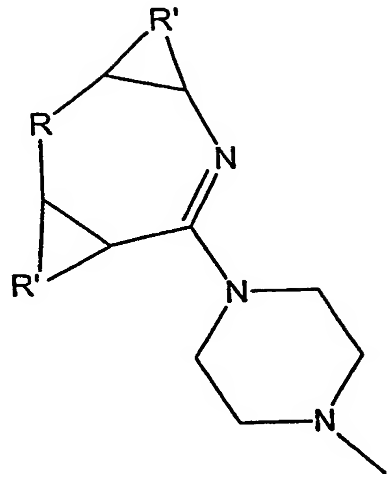
of antipsychotic drug, useful for the treatment of schizophrenia and/or bipolar disorder.

A further object of the present invention is to provide a method for identifying an agents as defined above.

Thus, in a first aspect of the present invention there is provided a pharmaceutical agent having serotonin 5-HT7 receptor antagonist activity and muscarinic m4 receptor agonist activity, for use in treating psychotic conditions such as schizophrenia and/or bipolar disorder, wherein the agent does not include compounds having a chemical structure falling within the following definition, namely:

bisarylazopines substituted at the azopine ring portion by a 4-methyl piperizinyl, wherein the aryl moieties are fused to the azopine ring and wherein aryl is phenyl, substituted phenyl or thienyl; including optional replacement of an azopine ring carbon atom with a nitrogen atom, or substitution of said ring carbon atom.

The compounds not encompassed by the present invention are represented by the following general formula:



wherein R represents substituted or unsubstituted C or N and each R' together with the carbon to which it is bonded independently represents phenyl, substituted phenyl or thienyl.

The above disclaimer is intended to exclude in particular any accidental anticipation by the compounds clozapine, fluperlipine and tenilapine. These compounds are three known antipsychotic drugs which display M4 agonism and 5HT7 antagonism as part of their wide spectrum of pharmacological actions. Thus, the compounds also show affinity for a large number of receptors, such as adrenergic α1, α2; histaminergic H1, H2, H3; dopaminergic D1, D2, D3, D4, D5; muscarinic cholinergic M1, M2, M3, M4, M5; serotonergic 5HT1A, 5HT2A, 5HT2B, 5HT2C, 5HT3, 5HT6, 5HT7. As such there is no suggestion in the art that the activities towards the M4 and 5HT7 receptors alone or together are significant or for that matter that the compounds are selective in their action i.e. do not act on many diverse receptors. Moreover, only the three compounds mentioned above out of the large number of atypical antipsychotic drugs show a very weak

agonist activity at muscarinic M4 receptors. While it has been suggested that M4 agonists or 5HT7 antagonists individually may have some therapeutic efficacy against the positive symptoms of schizophrenia, based on results in animal models (Bymaster et al., 1998; Shannon et al., 1999a,b; Pouzet et al., 2002), M4 agonists or 5HT7 antagonists individually have failed to show activity in animal models predictive of efficacy against the negative symptoms of schizophrenia (Bymaster et al., 1998; Pouzet et al., 2002). In view of the very large number of receptors potentially linked to the treatment of schizophrenia, which would include adrenergic α1, α2; histaminergic H1, H2, H3; dopaminergic D1, D3, D4, D5; muscarinic cholinergic M1, M2, M3, M4, M5; and serotonergic 5HT1A, 5HT2A, 5HT2B, 5HT2C, 5HT3, 5HT6, 5HT7 receptors, in addition to D2 receptors, there is no suggestion in the art that the specific combination of activities just towards the M4 and 5HT7 receptors is significant for the treatment of schizophrenia. Since most clinically useful atypical antipsychotic drugs do not show M4 agonist activity it is likely that the skilled artisan would not generally believe this property to be important clinically. It has never before been suggested, or demonstrated, that combining the properties of M4 agonism and 5HT7 antagonism, in the absence of any other pharmacological activity, would give activity against all the range of symptoms of schizophrenia.

As used herein the term agonist refers to a ligand that, upon binding to said receptor, triggers activation

of a chemical signalling cascade that results in a definable change in the behaviour or physical or biological state of a cell (including partial agonists which cause detectable but sub-maximal activation of signalling cascades) and the term antagonist refers to a molecule that, by virtue of binding to said receptor, is able to block the cell-activating influence of an agonist to said receptor, and which itself does not result in substantial activation of the cell.

The pharmaceutical agent may comprise a mixture of at least two compounds, wherein at least one of said compounds possesses serotonin 5-HT7 receptor antagonist activity and wherein at least one of said compounds possess muscarinic m4 receptor agonist activity.

Alternatively, the pharmaceutical agent may comprise a compound which possess both serotonin 5-HT7 receptor antagonist activity and muscarinic m4 receptor agonist activity, hereinafter termed a serominic compound.

Preferably the pharmaceutical agent additionally has a low or substantially no dopaminergic D2 receptor affinity.

A low dopaminergic D2 receptor affinity may be, for example, a minimum of at least 5 fold less than the affinity at the muscarinic m4 and/or serotonin 5-HT7 receptors.

More preferably the dopaminergic D2 receptor affinity is at least 5 fold, preferably at least 10 or 20 fold or at least 50 fold less than the affinity at the muscarinic m4 and/or serotonin 5-HT7 receptors.

Emerging evidence suggests that schizophrenia results from dysfunction of specific neural circuits in the brain. There is pathological evidence for dysfunction of cells in the prefrontal cortex in schizophrenic patients, along with clear indications from brain imaging studies that the prefrontal cortex is hypofunctional. The prefrontal cortex is a key part of the corticolimbothalamic circuit, which connects it both directly and indirectly to the midline thalamic nuclei. Dysfunction of this circuit in schizophrenia is consistent with the concept that the symptoms of schizophrenia are due to perturbation of midline thalamic function. Without wishing to be bound by theory, the present inventors therefore hypothesised that a pharmacological agent able to restore disturbed thalamic and prefrontal cortex function may effectively treat the symptoms of schizophrenia.

Muscarinic m4 receptors (Eglen, 2001) are located in brain regions that have been implicated in psychosis, including the prefrontal cortex, and are present in the specific neurones which are compromised in the post-mortem prefrontal cortex tissue from schizophrenic patients. While most APDs either have no affinity for the m4 receptor or act as antagonists, there is some evidence that m4 agonists may show APD-like activity in some tests. This is consistent with evidence that the levels of m4 receptors may be reduced in prefrontal cortex from schizophrenic patients as compared to normal controls (Crook et al., 2001). In addition, serotonin 5HT7

receptors (Vanhoenacker et al., 2000) are strikingly localised to thalamic nuclei. Some of the more effective atypical APDs have significant 5HT7 affinity as part of their complex pharmacological profile.

The present inventors therefore considered it possible that the combination of the two unusual properties - 5HT7 antagonist activity and muscarinic m4 agonist activity - might act to restore disturbed function in the brains of schizophrenic patients. Furthermore, they hypothesise that 5HT7 antagonist activity and muscarinic m4 agonist activity, in the absence of D2 affinity might be sufficient to bestow effective APD activity on such a pharmacological agent. According to this hypothesis, an agent possessing 5HT7 antagonist activity and substantial muscarinic m4 agonist activity, yet without significant D2 dopamine affinity, is postulated to show antipsychotic efficacy against both positive and negative symptoms. Such an agent may show an improved therapeutic profile relative to existing APDs, in terms of improved clinical efficacy and reduced side effect profile.

The present inventors have observed that some existing agents used in the treatment of schizophrenia have affinity for 5HT-7 and M4 receptors, as well as many other receptors. There is however no suggestion in the art that the activity of the agents is due to a combination of their affinity and/or activity on the 5HT-7 and M4 receptors. These agents fall under the general grouping of bisorylazopines and in order to avoid any

accidental anticipation by these compounds, such compounds are not encompassed by the present invention.

In addition, schizophrenic patients show marked deficits in cognitive tests, and this is thought to contribute to their inability to lead a relatively normal life. Since there is evidence that m4 muscarinic agonists should act as cognitive enhancers (Jerusalinsky et al., 1998), a drug with substantial m4 agonist activity may also be effective against the cognitive impairment characteristic of the disease.

Hence, the present inventors sought to demonstrate the potential therapeutic efficacy of a pharmacological agent combining selectivity versus other receptors with serotonin 5HT7 antagonist activity and muscarinic m4 agonist activity.

The compounds with properties according to the present invention may be provided as pharmaceutical formulations wherein the compound or compounds is/are admixed with a pharmaceutically acceptable carrier (e.g. binder, corrective, corrigent, disintegrator, emulsion, excipient), diluent or solibilizer to give a pharmaceutical composition by a conventional manner, which is formulated into, for example, a tablet, capsule, granule, powder, syrup, suspension, solution, injection, infusion, deposit agent, suppository. Administration may be for example orally or parenterally.

When the tablets are used for oral administration, typically used carriers include sucrose, lactose, mannitol, maltitol, dextran, corn starch, typical

lublicants such as magnesium stearate, preservatives such as paraben, sorbin, antioxidants such as ascorbic acid, α-tocopherol, cystein, disintegrators or binders. When administered orally as capsules, effective diluents include lactose and dry corn starch. A liquid for oral use includes syrup, suspension, solution and emulsion, which may contain a typical inert diluent used in this field, such as water. In addition, sweeteners or flavors may be contained.

In the case of parenteral administration such as subcutaneous injection, intraveneous injection, intramuscular injection, intraperitoneal injection or infusion, the pH of the active ingredient solution may be appropriately adequately adjusted, bufferized or sterilized. Examples of usable vehicle or solvent include distilled water, Ringer water and isotonic brine. For intraveneous use, the total concentration of solute is adjusted to make the solution isotonic.

Suppositories may be prepared by admixing the compounds of the present invention with a suitable nonirritative excipient such as those that are solid at normal temperature but become liquid at the temperature in the intestine and melt in rectum, such as cocoa butter and polyethylene glycols to release the active ingredient.

The dose can be determined depending on age, body weight, administration time, administration method, combination of drugs, the level of condition for which a patient is undergoing therapy, and other factors. While

the daily dose may vary depending on the conditions and body weight of patients, the species of active ingredient, and administration route, in the case of oral use, the daily dose is about 0.1 mg-100 mg/person/day, preferably 0.5 mg-30 mg/person/day. In the case of parenteral use, the daily dose is desirably 0.1 mg-50 mg/person/day, preferably 0.1 mg-30 mg/person/day for subsutaneous injection, intraveneous injection, intramuscular injection and intrarectal administration.

Accordingly the agents with properties according to the present invention, may be used in a method for treating psychotic disorders, for example schizophrenia for example the positive and/or negative symptoms of schizophrenia, or bipolar disorder.

The present invention accordingly provides agents with properties according to the present invention for use in medicine or therapy.

According to a second aspect of the present invention, there is provided use of the agents with properties according to the present invention for the preparation of a medicament for the treatment of psychotic disorders, for example, schizophrenia e.g. the positive and/or negative symptoms of schizophrenia and/or the cognitive deficits of schizophrenia, and/or bipolar disorder.

According to a third aspect of the present invention, there is provided a method of identifying an agent having the properties according to the present invention comprising the steps of:

a) providing an agent to be tested;

b) subjecting said agent to one or more test procedures to identify 5-HT7 receptor antagonist activity and muscarinic m4 receptor agonist activity of said agent;

wherein the desired agent is considered to have been identified when said agent provides a 5-HT7 receptor antagonist activity and a muscarinic m4 receptor agonist activity.

Desirably, the method further includes the step of subjecting the agent to a test procedure to identify low dopaminergic D2 receptor affinity.

More preferably the agent is generally more selective than existing antishizophrenic and/or anti-bipolar disorder drugs. That is the agent has less affinity for other receptors than existing antischizophrenic and/or anti-bipolar disorder drugs.

Thus, the method may further comprise the step of subjecting the agent to a procedure to detect affinity for other receptors namely adrenergic $\alpha 1$, $\alpha 2$; histaminergic H1, H2, H3; dopaminergic D1, D2, D3, D4, D5; muscarinic cholinergic M1, M2, M3, M4, M5; serotoneric 5HT1A, 5HT2A, 5HT2B, 5HT2C, 5HT3, 5HT6, 5HT7 and selecting agents which display affinity for less than 75% of said receptors, preferably less then 50% of said receptors.

The present invention will now be described by way of example with reference to the following experimental section and drawings in which:

Figure 1 is a representation of a full treatment paradigm of chronic PCP rat model with PTAC and SB258741;

Figure 2 shows the effect of haloperidol (Hal), clozapine (cloz) or the experimental serominic combination - PTAC + SB258741 (PTAC/SB) - on chronic PCP-induced hypofrontality;

Figure 3 relates to the reticular thalamic metabolic activity and shows the effect of haloperidol (Hal), clozapine (cloz) or the experimental serominic combination - PTAC + SB258741 (PTAC/SB) - on chronic PCP-induced hypoactivity;

Figure 4 relates to the auditory structure metabolic activity and shows the effect of haloperidol (Hal), clozapine (cloz) or the experimental serominic combination - PTAC + SB258741 (PTAC/SB) - on chronic PCP-induced hypoactivity.

Figure 5 - Effects of PTAC alone on apomorphine-induced deficits in PPI in rats. Values represent mean ± SEM. ##p<0.01 compared to Vehicle$^+$APO group and PTAC treated groups (Dunnett's test). n=7.PPI (average) means that PPI collapsed across all three prepulse intensities (73,75 and 80dB).

Figure 6 - Effects of SB258741 alone on apomorphine-induced deficits in PPI in rats. Values represent mean± SEM. ##p<0.01 compared to Vehicle$^+$Vehicle group (t-test). There are no significant difference between Vehicle$^+$APO group and SB258741 treated groups (Dunnett's test). n=7.

Figure 7 - Synergisitic effect of PTAC and SB258741 on apomorphine-induced deficits in PPI in rats. Values represent mean±SEM.##p<0.01 compared to Vehicle+Vehicle+Vehicle group (t-test). **p<0.01 compared to Vehicle+Vehicle+APO group (t-test). n=7.

In vivo activity:

To test the hypothesis that a serominic compound would show efficacy in the treatment of schizophrenia, we exploited our recent discovery of an animal model of schizophrenia that mimics the neurochemical and metabolic dysfunction in the brains of patients with schizophrenia (Cochran et al., 2003).

Schizophrenic patients show reduced metabolic activity in the prefrontal cortex, auditory system and hippocampus, along with reduced levels of expression of parvalbumin within inhibitory interneurones of the prefrontal cortex. The hypometabolism in the prefrontal cortex is not restored to normal by typical APDs, or by atypical APDs such as clozapine, although the hypometabolism in the auditory system is thought to be improved by both typical and atypical APDs (Schroeder et al., 1994; Andreasen et al., 1992 and Potkin et al., 1994). We have previously reported (Cochran et al., 2003) that these deficits observed in schizophrenic patients are reproduced in rats treated chronically with phencyclidine (PCP) - a drug known to cause schizophrenic symptoms when adminstered chronically in humans. We have

also observed that, in parallel with the clinical observations, the prefrontal cortex hypometabolism in PCP-treated rats is not attenuated by the representative atypical and typical antipsychotic drugs clozapine or haloperidol (Cochran et al., 2003), whereas the hypometabolism in the auditory system and hippocampus is restored towards normal levels by both haloperidol and clozapine. Thus evidence that a serominic compound could restore the prefrontal cortex hypometabolism in PCP-treated rats towards normal levels would indicate that a serominic compound would be more effective than currently available antipsychotic drugs for the treatment of schizophrenia. (5R,6R)6-(3-propylthio -1,2,5-thiadiazol-4-yl)-1-azabicyclo(3.2.1)octane.

When the m4 muscarinic partial agonist (PTAC) (Calbiochem Biochemicals) was administered chronically in combination with the 5HT7 antagonist SB258741, the drug combination was found to attenuate the hypometabolism in the prefrontal cortex, and thus demonstrate efficacy superior not only to haloperidol, but also to clozapine. A similar effect was observed in the reticular thalamus, which is a brain region functionally connected with the prefrontal cortex and involved in the regulation of its activity. In addition, the hypometabolism in the auditory system and hippocampus was also restored to normal by the M4 agonist/5HT7 antagonist combination. Thus, the combination of M4agonist/5HT7 antagonist appears to exert

profound antipsychotic activity, as assessed by these markers, in the absence of any D2 affinity.

Experimental procedure

Male hooded Long Evans rats (180-220g) were randomly allocated to one of the following treatment groups: vehicle/vehicle, PCP/vehicle, and PCP/SB258741+PTAC. The first drug was administered by i.p injection and the second drug was delivered via osmotic minipump which was implanted under halothane anaesthetic on day 8 of the YRING PCP model. See WO01/75440. The doses of drug used were 2.58mg/kg PCP, vehicle (sterile saline), 0.1mg/kg/day PTAC together with SB258741 20mg/kg/day. The full treatment paradigm of the chronic PCP model is shown in Figure 1.

On the day of the 2-DG procedure, the animals were prepared according to the method of Crane and Porrino, (1989). The brains were sectioned and exposed to X-ray film and LCGU measurements were calculated using the MCID 5 densitometry system. The results were analysed using a one way ANOVA followed by LSD post hoc test where appropriate for each discrete brain region. Statistical significance was defined as $p<0.05$.

The rats treated chronically with the M4agonist/5HT7 antagonist combination did not show any overt evidence of side-effects.

LCGU within cortical regions

The effect of PTAC and SB258741 (the serominic combination) on LCGU within cortical brain regions is shown in table 1.1. The only cortical brain region which showed a significant metabolic hypofunction induced by PCP was in the prefrontal cortex. Within the prelimbic region of the prefrontal cortex, a significant decrease in LCGU following chronic PCP compared to controls was observed in layer I (19%) and layers II and III (25%). Layers V&VI was just outside statistical significance. When SB258741+PTAC were administered in conjunction with PCP they reversed the PCP-induced hypofunction back to control levels (see table 1.1). The medial orbital cortex also displayed a significant decrease in LCGU following chronic PCP treatment compared to control animals within layer I (16%), layers II&III (19%) and layers V and VI (16%). No other cortical brain region showed any significant alterations in LCGU following any combination of drug treatment.

LCGU within auditory structures

Table 1.2 shows the effect of the serominic combination given in combination with the YRING PCP Model on LCGU in auditory brain structures. PCP treatment induced a metabolic hypofunction within a few structures of the auditory system. Within the ventral lateral lemniscus, the ventral cochlear nucleus and the primary auditory cortex chronic PCP treatment significantly reduced LCGU (26%, 21% and 25% respectively). In all

these three auditory structures the serominic combination reversed the PCP-induced hypofunction

LCGU within thalamic nuclei

The effect of the serominic given in combination with the YRING PCP Model on LCGU within thalamic brain regions is shown in table 1.3. The only thalamic nuclei which displayed a metabolic hypofunction with PCP was the reticular thalamus. Within the dorsal region of the reticular thalamus LCGU was significantly decreased by 25% and in the ventral reticular thalamus the PCP-induced decrease was 21%. In both regions of the reticular thalamus the serominic combination completely reversed the PCP-induced hypofunction.

	LCGU (µmol/100g/min)		
	Vehicle	PCP	
	vehicle	Vehicle	Serominic Combination
mO1	113±4	95±3*	98±4*
mO2	118±7	95±6*	112±5
mO3	111±8	93±3*	102±5
vO1	135±8	153±8	163±3
vO2	165±8	168±12	158±10
vO3	155±8	151±9	147±8
lO1	153±7	150±11	162±5

IO2	158±8	155±10	138±7
IO3	148±6	138±7	138±7
PrL1	129±1	105±5*	124±4
PrL2	144±6	108±4*	145±2
PrL3	148±7	128±8	143±7
IL1	97±5	98±6	100±5
IL2	99±5	102±7	99±5
IL3	96±5	96±7	97±6
M1	143±9	128±7	132±6
M2	124±9	117±4	119±2
Cg1	127±8	123±5	124±6
Cg2	138±8	135±6	132±4
Cg3	117±7	119±6	108±3
Pir	153±7	137±7	139±6
I	92±6	86±7	84±5
RS1	117±7	116±11	119±2
RS2	116±7	120±11	120±5
RS3	103±7	110±11	107±4
Ent1	79±6	81±4	83±5
Ent2	73±5	77±4	73±5
Ent3	69±4	72±5	63±8

Table 1.1 The effect of chronic SB258741+PTAC treatment on chronic PCP induced changes in LCGU within cortical region. All data expressed as mean LCGU (µmol/100g/min)

± SEM (n=5-7). * signifies p<0.05 compared to vehicle-vehicle treated animals, signifies p<0.05 compared to PCP-vehicle treated animals. The abbreviations used in the table are listed hereinafter.

	LCGU (µmol/100g/min)		
	Vehicle	PCP	
	vehicle	Vehicle	Serominic
AudCx1	177±7	133±4*	164±8
AudCx2	140±16	123±3	131±6
VisCx1	147±6	129±11	133±9
VisCx2	129±8	115±8	117±9
ILL	113±6	94±6	110±6
DLL	105±4	87±3	112±5
VLL	121±7	95±4*	114±5
VCP	129±8	95±4*	124±5

Table 1.2 The effect of chronic SB258741+PTAC treatment on chronic PCP induced changes in LCGU within auditory structures. All data expressed as mean LCGU (µmol/100g/min) ± SEM (n=5-7). * signifies $p<0.05$ compared to vehicle-vehicle treated animals, signifies

p<0.05 compared to PCP-vehicle treated animals. Appendix 1 details the abbreviations used in the table.

	LCGU (µmol/100g/min)		
	Vehicle	PCP	
	Vehicle	Vehicle	Serominic
AV	161±8	145±10	148±3
AM	144±8	138±8	139±5
Rt dorsal	107±6	80±5*	109±2
Rt ventral	115±7	91±5*	120±2
G	155±9	142±11	140±5
Re	107±8	118±7	115±4
Rh	106±3	102±6	110±3
VL	119±4	121±11	116±3
VM	137±10	137±13	134±4
PV	87±5	88±6	83±2
MD	133±10	131±8	133±4
CM	117±6	110±6	113±2
CL	122±8	129±10	127±2
IM	108±4	109±6	112±4

Table 1.3 The effect of chronic SB258741+PTAC treatment on chronic PCP induced changes in LCGU within thalamic nuclei. All data expressed as mean LCGU (µmol/100g/min)

±SEM (n=5-7). * signifies p<0.05 compared to vehicle-vehicle treated animals, signifies p<0.05 compared to PCP-vehicle treated animals. Appendix 1 details the abbreviations used in the table.

This study has shown that the chronic PCP-induced metabolic hypofunction in the prelimbic region of the prefrontal cortex is completely reversed back to control levels when administered with chronic PTAC and the serominic combination. This is of great interest as we have shown previously (Cochran et al., 2003) that both clozapine and haloperidol failed to reverse this PCP-induced metabolic hypofunction in the prelimbic region of the prefrontal cortex.

Local glucose utilisation was measured in the prelimbic area of the prefrontal cortex after chronic treatment with PCP alone or with the antipsychotic drugs. Note that the reduced metabolic activity caused by PCP (*p<0.05 vs control) is restored to normal values by the serominic combination (#p<0.05 vs PCP alone) but not by haloperidol or clozapine.

Thus, the combination of M4agonist/5HT7 antagonist appears to exert profound antipsychotic activity, as assessed by these markers, in the absence of any D2 antagonist activity. This provides dramatic evidence that an agent with serominic properties is likely to be

markedly superior to any of the currently-available antipsychotic agents.

This inability of haloperidol and clozapine to modulate the hypofrontality is consistent with data from clinical studies where similar results are obtained in medicated and unmediated patients (Schroeder et al., 1994; Andreasen et al., 1992 and Potkin et al., 1994). The prefrontal cortex is involved in certain types of working memory and has been implicated in the cognitive dysfunction observed in schizophrenic patients. Also this hypofunction has been correlated to the intensity of negative and cognitive dysfunction of schizophrenia (Wolkin et al., 1992: Schroder et al., 1995). There is conflicting evidence that clozapine and other new atypical antipsychotics are effective in treating these symptoms of the disease, but it is generally accepted that the negative symptoms and cognitive impairments seen in schizophrenia have proved very difficult to treat to date (Goldberg et al., 1993). In this study we have shown that the serominic combination can reverse the PCP-induced hypofunction in the PFC.

In the reticular nucleus of the thalamus the PCP-induced metabolic hypofunction is restored to control levels when the serominic combination is administered (Fig 3). Previously we have shown that both clozapine and haloperidol failed to reverse the PCP-induced

hypofunction in the reticular nucleus of the thalamus (Cochran et al., 2003).

Local glucose utilisation was measured in the ventral reticular thalamic nucleus after chronic treatment with PCP alone or with the antipsychotic drugs. Note that the reduced metabolic activity caused by PCP (*p<0.05 vs control) is restored to normal values by the serominic combination (#p<0.05 vs PCP alone) but not by haloperidol or clozapine.

The mechanism by which serominic is reversing the PCP-induced metabolic hypofunction in the reticular thalamus is postulated to be through a 5HT7 receptor mediated mechanism since 5HT7 receptors are concentrated in thalamic areas.

The fact that the serominic combination can reverse the PCP-induced hypofunction in the dorsal and ventral parts of the reticular thalamus is again of much interest as it suggests that the serominic may be beneficial in treating the positive symptom of the disease (poor filtering of irrelevant information) and also indirectly in treating the negative symptoms as the reticular thalamus has reciprocal projections to the prefrontal cortex. Once again, the serominc combination shows superior efficacy to current APDs.

In selected auditory brain structures (ventral lateral lemniscus, ventral cochlear nucleus and in the primary auditory cortex)chronic PCP treatment caused a significant hypofunction. Previously, we reported that both clozapine and haloperidol reversed the PCP-induced

hypofunction in these auditory structures (Cochran et al., 2003), consistent with their efficacy against positive symptoms such as auditory hallucinations. This study shows that the serominic is also effective in reversing the metabolic hypofunction within these auditory structures.

Local glucose utilisation was measured in the ventral lateral lemniscus after chronic treatment with PCP alone or with the antipsychotic drugs. Note that the reduced metabolic activity caused by PCP (*$p<0.05$ vs control) is restored to normal values by clozapine or the serominic combination (#$p<0.05$ vs PCP alone) but haloperidol only partially restores the hypofunction. Similar effects were observed in the auditory cortex and other auditory structures.

Decreased metabolism of the temporal lobe (auditory cortex and hippocampus) have been directly correlated with the positive symptoms of the disease (Buchsbaum et al, 1996; Klemm et al., 1996). Therefore this study shows that the serominic is effective in reversing the metabolic hypofunction within these auditory structures, which are associated with hallucinations (positive symptom of the disease). Therefore it appears that a serominic agent will behave in a similar way to clozapine and haloperidol in ameliorating the positive symptoms of the disease.

These results imply that a serominic may be beneficial in treating both the negative symptoms and cognitive impairment which to date have proved very difficult to treat, as well as being effective in treating the positive symptoms of the disease.

The startle reaction to a strong acoustic stimulus is reduced by the prior presentation of a weak stimulus. This reduction, termed prepulse inhibition (PPI), has been used as a measure of sensorimotor gating and significantly diminished in schizophrenic patients (Braff et al., 1978). In rats, the disruption of PPI by apomorphine is reversed by the administration of antipsychotics with potency correlating well with clinically effective dosages of each drug. Thus, the disruption of PPI by apomorphine is a valid animal model for some aspects of schizophrenia (Swerdlow et al., 1994).

Methods

Male Wistar rats (Japan SLC Inc., Shizuoka, Japan) were used. They were housed in a light, humidity and temperature controlled environment maintained on a 12-hour/12-hour light/dark schedule (light on at 7 am) with food and water provided ad libitum. Four startle chambers (SR-LAB, San Diego Instruments, San Diego, CA) were placed in a sound-attenuated room. Each chamber consisted of Plexiglas cylinder 8.8 cm in diameter resting on a 12.7 x 20.3 cm Plexiglas stand. Acoustic stimuli and background noise were presented via a

Supertweeter mounted 24 cm above the Plexiglas cylinder. Startle magnitude was detected and recorded by a microcomputer and interface assembly (San Diego Instruments) as transduced cylinder movement via a piezoelectric device mounted below the Plexiglas stand.

One day before drug testing, all rats were exposed to a "matching" startle session. Data from this session were used to assign rats to balanced groups according to their startle responses. On the drug testing day, rats were treated with vehicle (sterile saline) or drug (PTAC and/or SB258741) subcutaneously 25 minutes prior to apomorphine (0.5 mg/kg, s.c.) treatments. Immediately after apomorphine treatments, rats were placed into the startle chamber and a test session was started. Each session was approximately 20 minutes and consisted of 5 minutes of 70-dB background followed by five trial types, PULSE ALONE trial: a 120-dB 50 ms noise burst, PREPULSE trials which consisted of 20 ms noise bursts 3, 5, 10 dB above 70-dB background noise followed 100 ms by a 120-dB 40 ms noise burst, NOSTIM trial: 100 ms of response was recorded during periods where no stimulus was presented. Each trial presented in pseudorandom order every 15 seconds for a total 60 trials (12 trials each). The percentage PPI was defined as 100 - [(startle amplitude on PREPULSE trial/startle amplitude on PULSE ALONE trial) x 100].

Results

Apomorphine (0.5 mg/kg, s.c.) significantly reduced the PPI (Fig. 1,2,3). Neither PTAC nor SB25871 alone affect the disruption induced by apomorphine (Fig. 1 and 2, respectively). PTAC combined with SB25871 restored the apomorphine-induced disruption of PPI (Fig. 3).

The agents with properties according to the present invention are useful as a novel type of antipsychotic agent which are effective for both the positive and negative symptoms of schizophrenia, and which may cause less side effects of extrapyramidal motor disorder and the like and which may cause less serious side effects such as agranulocytosis and the like.

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Each of the following references is specifically incorporated herein by reference. In addition, one skilled in the art can rely on the contents of these references to make and use embodiments of this invention.

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Abbreviations

The abbreviations used in this specification are those used within the article Brain in Stereotaxic Coordinates (Paxinos and Watson, 1998)

1, layer I of cortex
2, layers II & III of cortex
3, layers V & VI of cortex

AM, anteromedial thalamus
Au1, primary auditory cortex
V, anteroventral thalamus
Cg (Cg1,-Cg3), anterior cingulate cortex
CM, centromedial thalamic nucleus
DLL, dorsal nucleus of the lateral lemniscus
Ent, entorhinal cortex
Ge, gelatinous nucleus of the thalamus
I, insular cortex
IL, infralimbic cortex
IM, intramedial thalamic nucleus
lO, lateral orbital cortex
M1 & M2, primary and secondary motor cortex
MD, mediodorsal thalamic nucleus
MG, medial geniculates

mO, medial orbital cortex

P, parietal cortex

Pir, piriform cortex

PrL, prelimbic region of the medial prefrontal cortex

PV, paraventriclular thalamic nucleus

Re, nucleus reuniens of the thalamus

Rh, rhomboid nucleus of the thalamus

RSG, retrosplenial cortex

Rt, reticular nucleus of the thalamus (d = dorsal part; v = ventral part)

V2, secondary visual cortex

VCP, ventral cochlear nucleus, posterior

VL, ventrolateral thalamic nucleus

VLL, ventral nucleus of the secondary auditory cortex

VM, ventromedial thalamic nucleus

vO, ventral orbital cortex

CLAIMS

1. A pharmaceutical agent having serotonin 5-HT7 receptor antagonist activity and muscarinic m4 receptor agonist activity, for use in treating psychotic conditions, the agent does not include compounds having a chemical structure falling within the following definition, namely:

bisarylazopines substituted at the azopine ring portion by a 4-methyl piperizinyl, wherein the aryl moieties are fused to the azopine ring and wherein aryl is phenyl, substituted phenyl or thienyl; including optional replacement of an azopine ring carbon atom with a nitrogen atom, or substitution of said ring carbon atom.

2. The pharmaceutical agent according to claim 1 wherein the psychotic condition is schizophrenia and/or bipolar disorder.

3. The pharmaceutical agent according to claim 1 or claim 2 which comprises a mixture of at least two compounds, wherein at least one of said compounds possess serotonin 5-HT7 receptor antagonist activity and wherein at least one of said compounds possess muscarinic m4 receptor agonist activity.

4. The pharmaceutical agent according to claim 1 or claim 2 which comprises a compound which possess both serotonin 5-HT7 receptor antagonist activity and muscarinic m4 receptor agonist activity.

5. The pharmaceutical agent according to any one of claims 1 to 4 which additionally has a low or substantially no dopaminergic D2 receptor affinity.

6. The pharmaceutical agent according to claim 5 wherein said dopaminergic D2 receptor affinity is a minimum of at least 5 fold less than the affinity at the muscarinic m4 and/or serotonin 5-HT7 receptors.

7. The pharmaceutical agent according to claim 6 wherein said dopaminergic D2 receptor affinity is at least 50 fold less than the affinity at the muscarinic m4 and/or serotonin 5-HT7 receptors.

8. A pharmaceutical formulation comprising a pharmaceutical agent according to any one of claims 1 to 7 together with a pharmaceutically acceptable carrier therefor.

9. Use of a pharmaceutical agent according to any one of claims 1 to 7 for the preparation of a medicament for the treatment or prophylaxis of schizophrenia and/or bipolar disorder.

10. A method of identifying an agent having the properties according to the present invention comprising the steps of:

a) providing an agent to be tested;

b) subjecting said agent to one or more test procedures to identify 5-HT7 receptor antagonist activity and muscarinic m4 receptor agonist activity of said agent;

wherein the desired agent is considered to have been identified when said agent provides a 5-HT7 receptor antagonist activity and a muscarinic m4 receptor agonist activity.

11. The method according to claim 10 further comprising the step of subjecting the agent to a test procedure to identify low dopaminergic D2 receptor affinity.

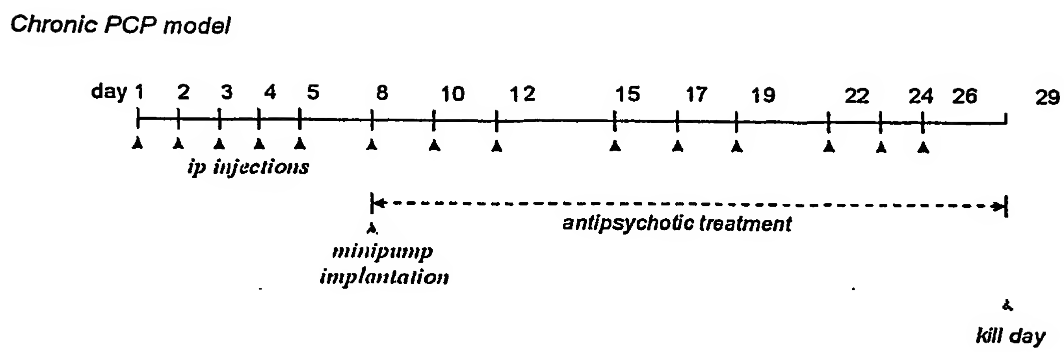

Figure 1

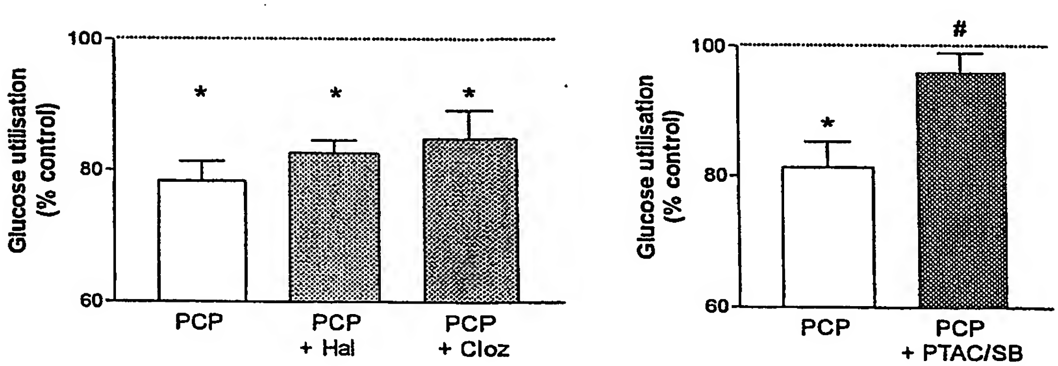

Figure 2

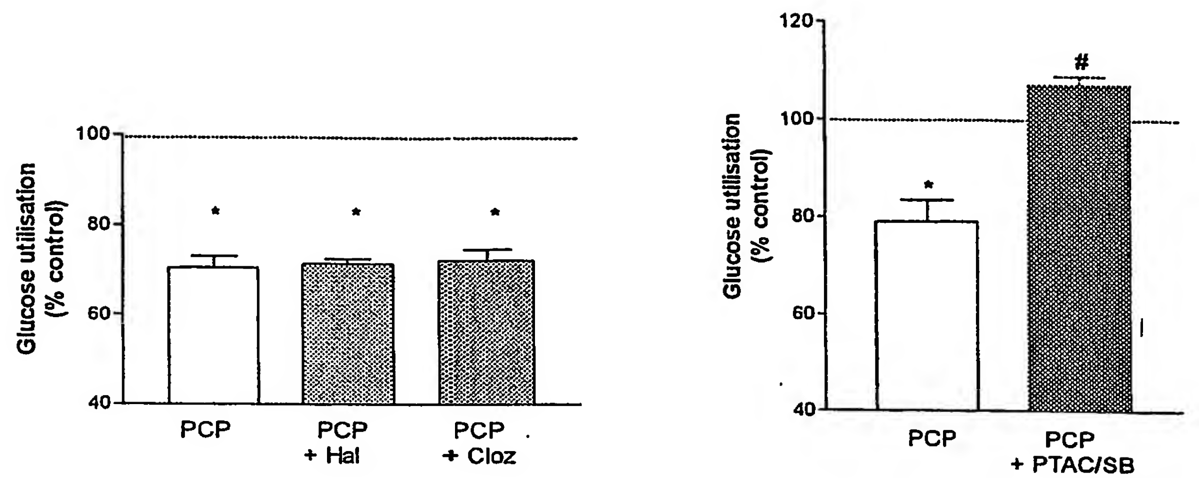

Figure 3

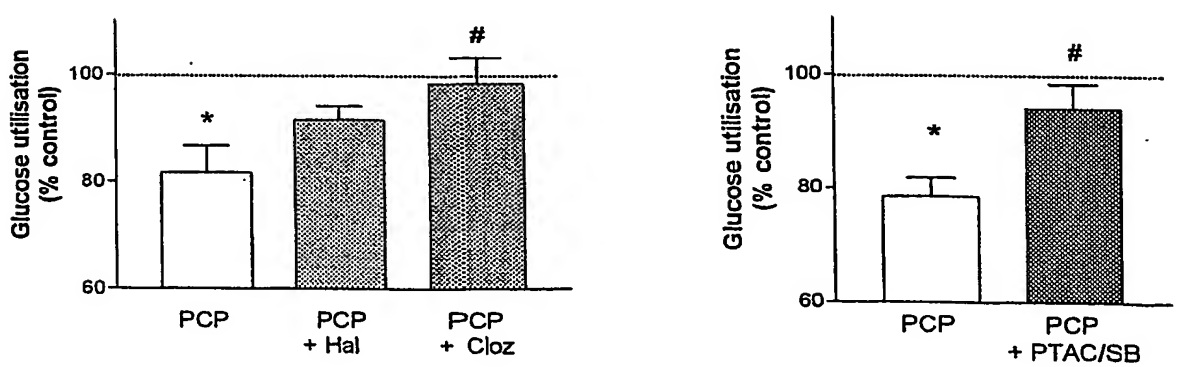

Figure 4

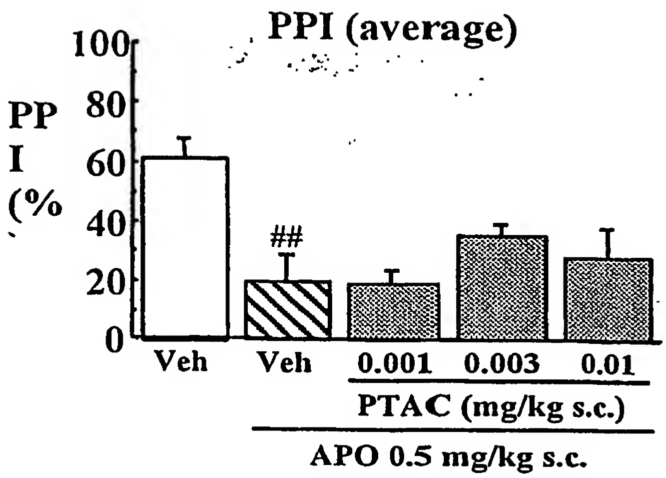

Figure 5

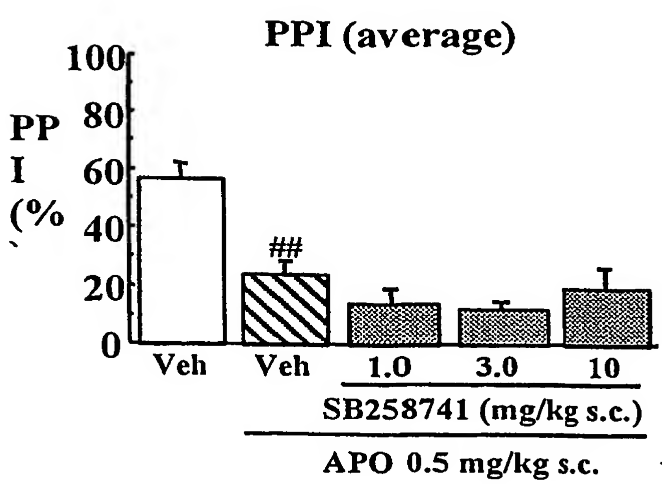

Figure 6

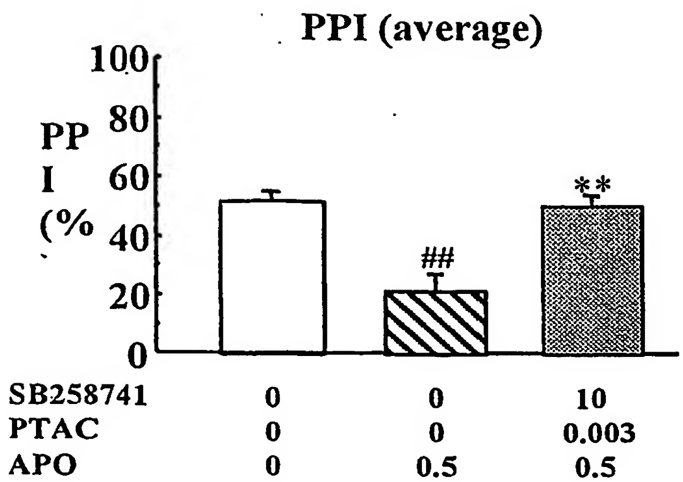
PPI (average)
PPI (%)
100
80
60
40
20
0
##
**
SB258741 0 0 10
PTAC 0 0 0.003
APO 0 0.5 0.5

Figure 7

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